Pharmacokinetics of imipenem in dogs

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Objective — To determine the plasma pharmacokinetics of imipenem (5 mg/kg) after single-dose IV, IM, and SC administrations in dogs and assess the ability of plasma samples to inhibit the growth of Escherichia coli in vitro.

Animals — 6 adult dogs.

Procedure — A 3-way crossover design was used. Plasma concentrations of imipenem were measured after IV, IM, and SC administration by use of high-performance liquid chromatography. An agar well antimicrobial assay was performed with 3 E. coli isolates that included a reference strain and 2 multidrug-resistant clinical isolates.

Results — Plasma concentrations of imipenem remained above the reported minimum inhibitory concentration for E. coli (0.06 to 0.25 µg/mL) for a minimum of 4 hours after IV, IM, and SC injections. Harmonic mean and pseudo-standard deviation half-life of imipenem was 0.80 ± 0.23, 0.92 ± 0.33, and 1.54 ± 1.02 hours after IV, IM, and SC administration, respectively. Maximum plasma concentrations (C_max) of imipenem after IM and SC administration were 13.2 ± 4.06 and 8.8 ± 1.7 µg/mL, respectively. Time elapsed from drug administration until C_max was 0.50 ± 0.16 hours after IM and 0.83 ± 0.13 hours after SC injection. Growth of all 3 E. coli isolates was inhibited in the agar well antimicrobial assay for 2 hours after imipenem administration by all routes.

Conclusions and Clinical Relevance — Imipenem is rapidly and completely absorbed from intramuscular and subcutaneous tissues and effectively inhibits in vitro growth of certain multidrug-resistant clinical isolates of E. coli. (Am J Vet Res 2003;64:694–699)

Imipenem is a carbapenem β-lactam antimicrobial that has been used widely in human and veterinary medicine. It has a broad spectrum of antimicrobial activity and is effective against most gram-positive, gram-negative, aerobic, and anaerobic organisms.1-3 It has been used to treat various infections in humans, including soft tissue, abdominal, respiratory tract, urinary tract, and bone infections,4,13 and to provide perioperative antimicrobial prophylaxis in abdominal and colorectal surgeries.4,13 In addition, imipenem is used commonly in humans when nosocomial infections are suspected or detected.16,17 Nosocomial infections are becoming increasingly problematic in veterinary medicine with the emergence of multidrug-resistant strains of bacteria.18-21

The pharmacokinetics of imipenem have been well established in human medicine.17,22-28 Both IV and IM administration routes are used in humans for treatment of nosocomial and serious infections.6,7,11,16,17 The use of imipenem in dogs has largely been extrapolated from the established dosage and pharmacokinetic data of humans. The pharmacokinetics of imipenem have been determined after IV administration in dogs,29 but neither the IM nor SC route has been thoroughly investigated.

The purpose of the study reported here was to characterize the pharmacokinetics of imipenem by use of high-performance liquid chromatography (HPLC) after single-dose IV, IM, or SC administrations (5 mg/kg) in dogs and to assess the ability of plasma samples to inhibit growth of Escherichia coli in vitro.

Materials and Methods

Dogs — Six 3- to 9-year-old sexually intact male cross-bred dogs weighing 19.6 to 58 kg (mean ± SD, 27.1 ± 7.1 kg) were used in this study.30 Dogs were housed at the University of Georgia Veterinary Medical Animal Care Facility. Animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A physical examination, CBC, serum biochemical profile, and urinalysis obtained by cystocentesis were performed on all dogs before starting the study. Urinalyses were repeated on the day of the first imipenem administration and the day after each additional administration. The CBC and serum biochemical profile were repeated 12 to 13 days after final administration.

Study design — The study was done in accordance with a randomized 3-way crossover design, with 3 dosing periods and a 1-week washout period between administrations. Imipenem (5 mg/kg)31 was administered IV, IM, and SC. The day prior to administration and sampling, the dogs were sedated with medetomidine (0.025 mg/kg, IV) for aseptic placement of an 18.5-gauge jugular catheter, which was used for blood sampling. An 18-gauge catheter was also placed in the cephalic vein of dogs scheduled for IV drug administration. Jugular catheters and cephalic catheters were flushed with 1 and 0.5 mL of saline (0.9% NaCl) solution, respectively, containing 5 U of sodium penicillin G.
The effect of medetomidine was reversed with atipamezole (0.1 mg/kg, IM) after catheters were placed.

Food was withheld for 24 hours before and 6 hours after imipenem administration. Water was not withheld at any time. Purified imipenem powder was reconstituted with sterile saline solution (11 mg/mL). In this study, imipenem was not combined with cilastatin. Imipenem was given as an IV bolus through the cephalic catheter for 30 to 60 seconds. The IM injection was divided into 4 equal volumes (2.0 to 3.5 mL/injection site) because of the large volume and administered in 4 sites (bilateral epaxial and gluteal musculature) to minimize discomfort. Subcutaneous injections were given at random intervals. Subcutaneous injections were not divided, because the total volume (8.0 to 14.0 mL/injection site) is well tolerated in adult dogs. Adverse reactions to injections were recorded.

Blood samples were collected from the jugular catheter immediately before and 0.08, 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 8, and 12 hours after drug administration. Sampling was a 4-step procedure. Blood (2.5 to 3.0 mL) was aspirated from the jugular catheter into a sterile 3-mL syringe. A 1.5-mL sample for drug analysis was collected and transferred into a heparinized polypropylene microcentrifuge tube. The initial blood sample was returned to the dog through the jugular catheter, and the catheter was flushed with 1 mL of heparinized saline solution. The heparinized blood samples were labeled and centrifuged immediately at 1000 X g for 10 minutes. Plasma was removed from the samples and combined with an equal volume of stabilizing solution (0.5M 3-morpholinopropanesulfonic acid-water-ethylene glycol [2:1:1]). The samples containing plasma in a stabilizing solution were vortexed, divided, and stored at –80°C for 90 days when samples are stored at ~80°C.

HPLC assay—The HPLC assay was performed with an established method. Briefly, plasma samples were subjected to ultrafiltration for 10 minutes at 6,000 X g by use of filter units, and 20 µL of the filtered sample was injected onto the HPLC column. Chromatography was performed by use of an HPLC system with a photodiode array detector. Chromatographic separation of the compounds was accomplished with a reversed-phase column (4 mm, 39 X 150 mm) and a mobile phase of 0.2M borate buffer (pH, 7.2) prepared with boric acid in HPLC-grade water at a flow of 1.5 mL/min. Absorbance was detected at 300 nm. Standard curves were prepared by adding known amounts of imipenem to blank dog plasma and using the same sample preparation procedure as described. Retention time of imipenem was 4.4 minutes. Standard curves were linear over a range of 0.1 to 100 µg/mL, and the limit of quantitation was 0.1 µg/mL. The interassay coefficient of variation for imipenem was < 8%, and the accuracy was > 96%.

Agar well antimicrobial assay—Three E coli isolates were used in this study, including a reference strain and 2 multi-drug-resistant isolates (2-2225 and 2-2359) cultured from wounds of dogs hospitalized at the University of Georgia Small Animal Veterinary Teaching Hospital. Both resistant isolates were determined to be susceptible to imipenem on the basis of standard in vitro antimicrobial sensitivity testing via the Kirby-Bauer method. The agar well antimicrobial assay was a modification of a previously described method.

Escherichia coli stock solutions were made in tryptose phosphate broth. Spectrophotometry was used to standardize the stock solutions. The reference strain was standardized to an absorbance of 0.389 at a wavelength of 600 nm. The stock solution was diluted serially in sterile water and plated on Luria Benton agar to determine the colony forming units (CFU)/mL in the stock solution (2.5 X 10^8 CFU/mL). This was repeated for the other organisms; 2-2225 was standardized at an absorbance of 0.394 and a wavelength of 600 nm with 2.8 X 10^8 CFU/mL, and 2-2559 at 0.382 with 2.9 X 10^8 CFU/mL. The stock solutions of bacteria in tryptose phosphate broth were labeled, divided into 1-mL aliquots, and stored at 4°C for no longer than 30 days.

Mueller-Hinton agar was prepared according to standard directions, autoclaved, and cooled to 36°C in a water bath. Two milliliters of bacterial stock was added to 200 mL of concentration agar and mixed thoroughly as a single large portion. Forty-three milliliters of seeded agar was poured into a 150-mm, 15-mm-deep sterile plate. Plates were allowed to cool and solidify. Using a standardization template, holes were punched in the agar with a sterile 4-mm biopsy punch. Punched holes of agar were removed with an adapted suction device. The resulting wells were filled with the designated samples.

Control plates were prepared in duplicate with each of the E coli isolates. The wells were filled with 40 µL of known concentrations of imipenem reconstituted in either plasma or PBS solution (PBSS); concentrations varied from 0 (negative control) to 0.39 mg/mL. These concentrations were chosen on the basis of known minimum inhibitory concentrations (MICs) of imipenem for the reference strain of E coli (0.06 to 0.25 µg/mL). Plates were incubated for 18 hours at 37°C. Each zone of inhibition was measured twice with standard calipers, and the mean of these 2 results was recorded.

To test plasma samples from dogs, plates were prepared in duplicate as described, and the wells were filled with the designated test samples collected from the dogs after imipenem administration. Each of the E coli isolates was tested separately. Two negative control wells were included on each plate: 1 with PBSS alone and the other with plasma from a dog that had not been treated with imipenem. Positive control wells contained imipenem, which directly dissolved in dog plasma and PBSS at concentrations of 0.01 and 0.03 mg/mL for each solution. Plates were incubated, and the zones of inhibition were measured.

Data analyses—The plasma concentrations of imipenem detected at various collection times were analyzed with non-compartmental methods by use of computer software. The terminal elimination rate constant (λ) was obtained from the slope of the least squares linear regression fit of the logarithms of measurable concentrations versus time in the log-linear terminal phase of the curve. The area under the plasma concentration-time curve (AUC) from time zero to the last observed concentration (4 hours) was determined by the linear trapezoidal rule, and the AUC was extrapolated to time infinite by dividing the last measurable plasma concentration by λ. Harmonic means and pseudo-standard deviations were calculated for half-life (t1/2). The maximum plasma concentrations (Cmax) of imipenem and times to reach maximum plasma concentration (Tmax) were observed values. The bioavailabilities after IM and SC administration were calculated by the ratio of the AUC from IM and SC administration to AUC from IV administration. Statistical analyses comparing pharmacokinetic values determined for various administration routes were performed by use of a 2-way ANOVA to test the effect of administration route, individual dog, and study period. Significance was defined as P < 0.05.

Standard curves were graphed for each bacterial isolate by use of known imipenem concentration versus zone of inhibition. Nonlinear regression of these curves was performed with commercially available computer software. The resulting equations describing the standard curves were used to convert zone of inhibition measurements from the dog plasma samples to imipenem concentrations. Times after
injection versus imipenem concentrations were graphed. Paired t-tests were performed at each time point to compare the imipenem concentration for the reference strain of E coli with clinical isolates 2-2225 and 2-2559. Significance was defined as \( P < 0.05 \).

Results

Pharmacokinetics—Pharmacokinetic parameters of imipenem after single IV, IM, and SC administrations were determined (Table 1). Plasma imipenem concentrations after IV administration declined rapidly with a terminal harmonic mean \( t_{1/2} \) of 0.80 ± 0.23 hours (Fig 1). Peak plasma concentrations were achieved rapidly after IM or SC administration, and terminal \( t_{1/2} \) was lengthened, compared with IV administration. There were no significant differences in AUC values among IV, IM, and SC administration. The MIC of imipenem for E coli (0.06 to 0.25 mg/L) was exceeded for at least 4 hours after IV, IM, and SC administration.

In vitro inhibition of bacterial growth—The shapes of the standard curves were consistent with a 2-parameter hyperbola. Nonlinear regression of these curves described the line of best fit as:

\[
y = a \cdot \frac{x}{b + x}
\]

where \( y \) is zone of inhibition, \( a \) is maximum inhibition, \( x \) is imipenem concentration, and \( b \) is the concentration of imipenem inhibiting 50% of bacterial growth. Correlation coefficients for the standard curves of the 3 bacterial isolates ranged from 0.98 to 0.99. The constants (\( a, b \)) predicted for each standard curve were similar to the actual calculated values (with a probability of this similarity being due to chance alone of < 0.002), and the SE around the constants was low.

Zones of inhibition generated by agar well antimicrobial assays were converted to imipenem concentrations by use of the standard curves. Graphs of times after injection versus imipenem concentrations for all administration routes were similar to those drawn from HPLC data (Fig 1). The last plasma collection time point after IV or IM administration that yielded serum and inhibited bacterial replication was 2.0 hours. A small zone of inhibition was evident from samples obtained 4 hours after SC administration for several dogs: 3 with the reference strain, 3 with the 2-2225 isolate, and 2 with the 2-2559 isolate. No significant differences were detected between the reference strain of E coli and either of the multidrug-resistant clinical isolates when comparing concentrations of imipenem determined by the agar well antimicrobial assay.

Response to injection—An adverse effect associated with imipenem administration was vocalization (IM route, 2/6 dogs; SC route, 1/6 dogs) during injection. Hypersalivation (2/6 dogs) was observed during rapid IV administration. No other adverse reactions, either immediate or delayed, were noticed during imipenem administration or at the local injection sites.

Blood analyses and urinalyses—For 5 of the 6 dogs, no abnormalities were detected via the original

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Administration route</th>
<th>IV</th>
<th>IM</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (mg·h/L)</td>
<td></td>
<td>20.2 ± 3.4</td>
<td>28.5 ± 12.6</td>
<td>30.6 ± 9.2</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td></td>
<td>0.80 ± 0.23</td>
<td>0.92 ± 0.33</td>
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<tr>
<td>CL (L/h/kg)</td>
<td></td>
<td>0.26 ± 0.06</td>
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<td>NA</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td></td>
<td>0.32 ± 0.04</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>C(_{max}) (mg/L)</td>
<td></td>
<td>NA</td>
<td>13.2 ± 4.06</td>
<td>8.8 ± 1.7</td>
</tr>
<tr>
<td>T(_{max}) (h)</td>
<td></td>
<td>NA</td>
<td>0.50 ± 0.16</td>
<td>0.83 ± 0.13</td>
</tr>
<tr>
<td>F (%)</td>
<td></td>
<td>NA</td>
<td>146 ± 68</td>
<td>159 ± 65</td>
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</tbody>
</table>

\*Harmonic mean ± pseudostandard deviation.

AUC = Area under the curve, \( t_{1/2} \) = Half-life, CL = Total body clearance, Vss = Steady state volume of distribution, \( C_{max} \) = Maximum plasma concentration, \( T_{max} \) = Time until \( C_{max} \) reached after drug administration, F = Bioavailability, NA = Not applicable.
CBC and serum biochemical profile. One dog had slightly increased alanine transaminase (ALT) activity of 130 U/L (reference range, 12 to 108 U/L). This dog had a persistently increased ALT activity 12 (169 U/L) and 30 (172 U/L) days after the study. One dog had increased ALT (120 U/L) and alkaline phosphatase (ALP) activities (278 U/L; reference range, 13 to 122 U/L) 12 days after the study was completed, but these abnormalities resolved 1 month after the study. Mild eosinophilia was seen in 2 dogs with eosinophil counts of 1,332 and 1,610 cells/mL, respectively, (reference range, 1 to 1,300 cells/mL) 12 to 13 days after the last administration of imipenem.

All dogs had results of urinalysis within reference ranges before the study. Various changes were detected after administration of imipenem. Sediment analysis in 1 dog revealed 10 to 50 WBCs/hpf with < 5 RBCs/hpf the day after an SC injection of imipenem. Subsequent urinalyses in this dog revealed < 5 WBCs/hpf in each additional sample. A urinary tract infection (UTI) was detected in another dog via urinalysis before the second of 3 administrations of imipenem. Clinical signs consistent with UTI were not detected in this dog. An Enterococcus sp was cultured that had in vitro susceptibility to amoxicillin-clavulanic acid was cultured by use of the Kirby-Bauer method. The dog was treated with a 3-week course of amoxicillin-clavulanic acid* (22 mg/kg, PO, q 8 h for 21 days) after completion of the imipenem study. Bacteriologic culture of urine 1 week after discontinuing treatment with amoxicillin-clavulanic acid resulted in no bacterial growth. Hyaline casts (1/hpf) were detected in 3 of 6 dogs the day after imipenem administration by various routes.

Discussion

This study determined that the MIC of imipenem for E coli could be achieved for at least 4 hours after IV, IM, or SC administration. In addition, growth of 1 reference strain and 2 multidrug-resistant clinical isolates of E coli was inhibited in the agar well antimicrobial assay for 2 hours after imipenem administration by all routes studied. To the authors’ knowledge, this is the first report of pharmacokinetic parameters of SC administration of imipenem to any species. In veterinary medicine, the ability to give continued injectable antimicrobials effectively by the SC route can make the difference between dogs remaining hospitalized or receiving continued care at home.

The t1/2 and AUC calculated after IV administration of imipenem were similar to published values. The t1/2 of imipenem after an IV bolus of 5 mg/kg to dogs was reported to be 0.51 hours.26,30 and the AUC was found to be 13.4 mg/L × hours.26 Following IV administration of imipenem in our study, plasma concentrations of the drug declined rapidly. However, after both IM and SC administrations, ‘flip-flop’ kinetics were observed in which the terminal t1/2 reflected the absorption rate rather than the elimination rate. Absorption of imipenem after IM or SC administration was characterized by a rapid burst effect followed by a sustained release of the drug, resulting in fairly constant plasma concentrations during a 2-hour period. The AUCs for imipenem administered by IM and SC routes were comparable to that for IV administration, indicating complete absorption of the drug. Thus, IM or SC administration of imipenem yields more desirable plasma concentration profiles than does IV administration.

Imipenem administration was found to be safe and well tolerated. Adverse effects in humans are rare but include nausea, vomiting, diarrhea, skin rashes or pruritus, and pain associated with IM injection.24,39 Nausea or vomiting is most commonly seen after rapid IV injection and is usually alleviated when a slow infusion is given.39 Hypersalivation during IV injection in 2 of our dogs may have indicated transient nausea. Possible signs of pain associated with injection in our dogs included vocalization during IM or SC administration. It is possible that discomfort in these dogs was partially related to the large volume of solution administered (approx 12 mL/dog); however, the drug itself may cause pain as well. Recommendations by the manufacturer for reconstitution of the commercially available product for IM use in humans, which is a combination of imipenem and cilastatin sodium, include a low-percentage lidocaine diluent for local analgesia.

In our study, imipenem was not combined with cilastatin as it is in commercially available products for humans. Renal tubular dipeptidases metabolize imipenem to inactive metabolites. Cilastatin is a dipeptidase inhibitor. Therefore, cilastatin is combined with imipenem to increase urinary excretion of active imipenem, thereby making it a more effective drug for UTIs.24,29 Addition of cilastatin also prevents proximal renal tubular necrosis, which has been seen in some susceptible animals after high doses of imipenem were administered alone.41-42 The inclusion of cilastatin with imipenem has little to no effect on the pharmacokinetics of imipenem and does not change its plasma t1/2 in humans.27 Therefore, it is unlikely that the lack of cilastatin in our study affected the pharmacokinetic values for imipenem in dogs. It would be beneficial to perform pharmacokinetic studies in dogs with the commercially available product in order to determine whether it can be used effectively IM and SC in dogs.

For clinical use, imipenem is only available commercially combined with cilastatin. Preparations for IV* (250- and 500-mg vials) or IM* (500-mg vial) administration are available for humans. The cost of these products is approximately $31/500-mg vial. Once reconstituted, commercial preparations of imipenem-cilastatin can be stored for no longer than 24 hours at 4°C.40 The combination product is a safer option for dogs with renal insufficiency, because the cilastatin should protect against renal tubular damage. A dose reduction is recommended in humans with renal failure, and this should apply to treatment of dogs as well.24,39

Mild changes in blood parameters reported in humans receiving imipenem include transient increases in liver enzyme activities, eosinophils, and platelets.38,39 In our study, 1 dog with preexisting high ALT activity had a persistent increase in ALT activity 12 days after administration of imipenem. It is unlikely that the increased liver enzyme activity in that dog was due to imipenem administration,
because the abnormality was present before and remained for 1 month after the study. Another dog had increased ALT and ALP activities 12 days after the third dose of imipenem, but liver enzyme activities returned to reference ranges 1 month after completion of the study. Based on the transient nature of the increased liver enzyme activity and its occurrence immediately after the study, the most likely cause was imipenem, metedomidine, or atipamezole. To the authors’ knowledge, there have been no reports of metedomidine or atipamezole causing increased liver enzyme activities. In humans, increased ALT and ALP activities associated with imipenem-cilastatin are usually transient and not associated with clinical abnormalities. However, hyperbilirubinemia and clinical icterus have been documented in humans after administration of imipenem-cilastatin.38,39 None of the dogs in our study developed clinical icterus.

Two of 6 dogs had eosinophilia 12 days after the last dose of imipenem administration. A CBC was not repeated to determine whether this abnormality resolved, and the importance of this delayed finding is not known. Transient eosinophilia has been reported in humans after administration of imipenem-cilastatin and is not associated with clinical problems.38,39 Other causes of eosinophilia could have been present in these dogs. All dogs were tested for heartworm disease and were receiving heartworm preventative medication. A fecal examination was not performed on the 2 dogs that developed eosinophilia. Although no abnormal skin lesions were seen, allergic skin disease was also not ruled out. Increases in platelet counts were not detected in any of the dogs in this study.

Mild abnormalities were also detected in some urine samples. Casts and WBCs in urine samples collected 24 hours after administration of imipenem may indicate mild renal inflammation secondary to imipenem administration. It is possible that this was seen because of using imipenem without cilastatin. Alternatively, the casts were low in number and may not be clinically important. One dog developed a UTI during the study. Clavamox administration successfully treated the Enterococcus infection. Although the UTI may have been related to imipenem-induced renal inflammation, it seems unlikely because few WBCs were consistently seen on urinalysis, and clinical signs and results of laboratory analyses never suggested systemic involvement or involvement of the upper portion of the urinary tract. An exhaustive workup to determine cause of the UTI was not done. The development of the UTI may have been related to a number of other factors as well. Some transient urinary retention occurred during the study in dogs hesitant to urinate while walked on a leash. Also, all dogs in the study were 3- to 9-year-old sexually intact male dogs, so subclinical prostatic infection would have to be considered.

Imipenem is an antimicrobial that has many indications for use in veterinary medicine. As nosocomial and multidrug-resistant infections in dogs become more common, the need for antimicrobials with a wide spectrum of activity, such as imipenem, increases. The tissue distribution of imipenem in dogs has not been reported and would be helpful for prescribing adequate dosages for achieving MICs in various tissues.

References


